

Claims:

1. A process for the preparation of an erythropoietin (EPO) from a cell or tissue in an in vitro system, comprising the steps of:

(a) providing

5 (i) at least one first cell or tissue, capable of inducing EPO production in a second cell or tissue, and

(ii) at least one second cell or tissue capable of producing EPO;

10 (b) culturing the first cell or tissue (i) and the second cell or tissue (ii) in an in vitro system under conditions and for a time suitable to induce EPO production and to express, produce and secrete EPO into the culture medium; and

15 (c) isolating the EPO produced from the culture medium.

2. A process according to claim 1 wherein the EPO is a natural or modified EPO.

3. A process according to any one of the preceding claims, wherein the first cell or tissue (i) is stimulated to induce the 20 production of EPO in the second cell or tissue (ii).

4. A process according to any one of the preceding claims, wherein the first cell or tissue (i) is stimulated by physical stimulation including electrical stimulation.

5. A process according to any one of the preceding claims, wherein the first cell or tissue (i) is stimulated by chemical stimulation including stimulation with at least one chemical compound.
- 5 6. A process according to any one of the preceding claims, wherein the first cell or tissue (i) is stimulated by reduced oxygen (O_2) partial pressure.
7. A process according to any one of the preceding claims, wherein the induction of the production of EPO in the second cell or tissue (ii) is mediated by a soluble or diffusible factor released by the first cell or tissue (i).
- 10 8. A process according to any one of the preceding claims, wherein the first cell or tissue (i) is stimulated to induce the production of EPO in the second cell or tissue (ii).
- 15 9. A process according to any one of the preceding claims, wherein the first cell or tissue (i) is identical to the second cell or tissue (ii).
10. A process according to any one of the preceding claims, wherein the first cell or tissue (i) and the second cell or tissue (ii) are selected from the same cell type, wherein the first cell or tissue (i) originates from a first host and/or first species and the second cell or tissue (ii) comprises or consists of cells originating from a second host and/or second species, and wherein the first host and/or first species is different from the second host and/or second species.
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11. A process according to any one of the preceding claims, wherein the first cell or tissue (i) is selected from a first cell type and the second cell or tissue (ii) comprises consist of or is selected from a second cell type, wherein the first cell type
5 is different from the second cell type.
12. A process according to any one of the preceding claims, wherein the second cell or tissue (ii) is of one cell type or of different cell types.
13. A process according to any one of the preceding claims, 10 wherein the first cell or tissue (i) and/or the second cell or tissue (ii) are selected from the group consisting of organ cultures, primary cells or cultured primary cells, derived from kidney including kindney from an autologous donor, liver, blood cells including lymphocytes, and erythrocytes, bone marrow and/or haematopoietic cells of the human or animal body and/or progenitor cells thereof, immortalised mammalian cell 15 lines including CHO, BHK, LLC-PK₁, COS, and mixtures and/or co-cultures of at least two cell types thereof.
14. A process according to any one of the preceding claims, 20 wherein the first cell or tissue (i) and/or the second cell or tissue (ii) comprise at least one recombinant cell or consist thereof.
15. A process according to claim 13 wherein the recombinant cell is transformed with at least one recombinant nucleic acid 25 molecule encoding EPO or derivates thereof.

16. A process according to claim 15 wherein the recombinant nucleic acid molecule codes for EPO with a glycoform profile typical for human, horse, bird, dog, or camel, respectively.
17. A process according to claim 15 or 16 wherein the nucleic acid sequence encoding the EPO is under control of at least one promoter and/or expression control element.
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18. A process according to claim 17 wherein the expression control element is an oxygen responsive element.
19. A process according to any one of the preceding claims, wherein in the in vitro system the culturing the first cell or tissue (i) and the second cell or tissue (ii) takes place in a shared cell culture compartment.
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20. A process according to any one of the preceding claims, wherein in the in vitro system the culturing of the first cell or tissue (i) and the second cell or tissue (ii) takes place in at least two separate cell culture compartments, wherein in a first compartment the first cell or tissue (i) is cultured and the second cell or tissue (ii) is cultured in at least one other compartment.
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21. A process according to any one of the preceding claims, wherein the in vitro system comprises at least one support for first cells or tissue and/or second cells or tissue, one or more cell culture compartments and a culture medium.
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22. A process according to any one of the preceding claims, wherein the support is connected or borders at least one side to the cell culture compartment.
23. A process according to any one of the preceding claims, 5 wherein the cell culture compartment is suppleable with liquid culture medium.
24. A process according to any one of the preceding claims, wherein the culture medium contains serum or is serum-free.
25. A process according to any one of the preceding claims, 10 wherein the cell culture compartments are separated from each other by a barrier, which inhibits cell migration from one compartment to another compartment, but allows the migration or diffusion of molecules from at least one compartment to another compartment.
26. A process according to any one of the preceding claims, 15 wherein the in vitro system includes at least one gas compartment which is suppleable with a gas or gas mixture.
27. A process according to any one of the preceding claims, 20 wherein the gas compartment is connected with, is corresponding with or borders to at least one of said cell culture compartments, such that at least one gas diffuses across the connection or border between the gas compartment and the cell culture compartment.
28. A process according to any one of the preceding claims, 25 wherein the gas compartment is connected with, is corre-

sponding with or borders to at least one culture medium supplied to at least one of said cell culture compartments.

29. A process according to any one of the preceding claims, wherein in at least one cell culture compartment a different culture medium and/or a different partial pressure of at least one gas is contained in comparison to another cell culture compartment.
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30. A process according to any one of the preceding claims, wherein at least two cell culture compartments are supplied with different gas or gas mixtures.
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31. A process according to any one of the preceding claims, wherein in step (b) the culturing is performed under a condition of reduced partial pressure of oxygen prevailing in at least one cell culture compartment.
- 15 32. A process according to claim 28, wherein the condition of reduced partial pressure of oxygen is prevailing in at least one cell culture compartment for an interrupted period of time.
- 20 33. A process according to claim 28, wherein the condition of reduced partial pressure of oxygen is prevailing in at least one cell culture compartment for a period of time sufficient to induce or increase the production and/or release of EPO.
34. A process according to claim 28, wherein the partial pressure of oxygen is normal or increased in another compartment.

35. A process according to any one of the preceding claims, wherein at least one culture medium comprises one or more growth factor or cytokine selected from the group consisting of granulocyte-macrophage colony stimulating factor (GM-CSF), 5 IL-3, granulocyte colony stimulating factor (G-CSF), transformin growth factor- β (TGF- β), platelet derived growth factor (PGF), insulin like growth factor (IGF), acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), hepatocytic growth factor (HGF), 10 keratocyte growth factor (KGF), and neural growth factor (NGF), in particular GM-CSF, IL-3, and G-CSF.

36. A process according to any one of the preceding claims, wherein the cells are cultured in monolayers.

37. A process according to any one of the preceding claims, 15 wherein the in vitro system is an artificial organ or an organotypic culture.

38. A process according to any one of the preceding claims, wherein the support is in form of a three-dimensional matrix or scaffold.

20 39. A process according to any one of the preceding claims, wherein the support comprises or consists of collagen, alginic acid, cellulose, polyhydroxyalkanoate, proteoglycans, agarose, gelatin, hyaluronan, or derivatives thereof, as well as synthetic polymers including PTFE, vicryl-polydioxanone-copolymers, polyglycolic acid, polyalkylene glycol-aromatic 25

polyester-copolymers, and PE, or a composite of different materials thereof.

40. A process according to any one of the preceding claims, wherein the support is in solid form, in particular fibrous or porous form including sponges, foams, porous fabrics, or in gel form.

5 41. A process to produce EPO in high purity, a subpopulation of EPO glycoforms, an individual EPO glycoform, or a mixture of at least two EPO glycoforms, comprising the steps:

10 (a) to (c) according to any one of the preceding claims,
and
(d) at least one further purification step selected from reversed phase HPLC, HPLC, immunoaffinity chromatography, immunoaffinity magnetic beads, cation and anion exchange chromatography, hydrophobicity chromatography, hydroxylapatit chromatography, dye affinity chromatography, lectin matrix purification, dihydroxybromyl matrix purification, gel filtration, salting out, precipitation with ammonium sulfate, isoelectric focussing, and a combination
15 thereof.

20 42. EPO produced by the process according to any one of claims 1 to 39.

43. EPO according to claim 42 comprising or consisting of a subpopulation of glycoforms.

44. EPO according to claim 42 comprising or consisting of an individual glycoform.
45. EPO according to any one of claims 42 to 43 being substantially free of human or animal blood products such as serum albumin.
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46. EPO according to any one of claims 42 to 45 being human EPO, equine EPO, canine EPO, avian EPO, or an recombinant EPO.
47. EPO according to any one of claims 42 to 46 produced by further processing EPO and forming a conjugate of EPO wherein EPO is covalently linked to polyethylene glycol.
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48. EPO according to any one of claims 42 to 47 that has been further modified to reduce immunogenicity and/or to prevent adverse effects of an immune response upon administration.
- 15 49. Pharmaceutical composition comprising EPO according to any one of claims 42 to 48 and one or more pharmaceutically acceptable excipients and/or further compounds.
50. Pharmaceutical composition according to claim 49 wherein the pharmaceutically acceptable excipient is selected from the group consisting of inorganic salts, pH buffers, amino acids, polyols, diluents, solvents, carriers, stabilisers, solubilisers, emulsifiers, preservatives, non-ionic detergents, surfactants, tonicity agents, anti-oxidants, and adjuvants.
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51. Pharmaceutical composition according to claim 50 wherein
the pharmaceutically acceptable excipient is selected from the
group consisting of sodium chloride, glucose, citrate, acetate
and phosphate buffered systems, urea, human, equine or bo-
vine serum albumin, lecithin, polyethylene glycol, mannitol,
sorbitol, benzyl alcohol, ethanol, parabens, phenols, cresol,
polysorbate 80, polysorbate 20, pluronic F68, glycine, me-
thionine, vitamin C, vitamin A, vitamin E.
52. Pharmaceutical composition according to any one of claims
49 to 51 wherein the compound is selected from the group
consisting of amino acids, polyols, antioxidants, vitamins,
trace elements, iron, anti-tumor agents, antineoplastic agents,
antiproliferative agents, cytostatica, anti-apoptotic agents, tox-
ines, enyzmes, diagnostic imaging or contrast agents, dyes,
15 antibacterial agents, antifungal agents, antiviral agents, cy-
tostatics, immunosuppressive agents, analgesic agents, hor-
mones, anti-inflammatory agents, and haematopoietic agents.
53. Pharmaceutical composition according to any one of claims
49 to 52 being an aqueous formulation, lyophilised, or spray
20 dried.
54. Use of EPO according to any one of claims 42 to 48 for ther-
apeutic and/or prophylactic treatment of diseases curable with
EPO.
55. Use of EPO according to any one of claims 42 to 48 for ther-
apeutic and/or prophylactic treatment of
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(a) diseases in connection with anaemia, including nephrogenic anaemia such as CRF related anaemia;

(b) anaemia secondary to treatment with anti-viral drugs, anti-proliferative drugs, anti tumor agents, antineoplastic agents, and immunosuppressive agents;

(c) anaemia secondary to treatment of HIV infection,

(d) anaemia secondary to chemotherapeutic or radiation regimens including chemotherapy and radiation therapy in connection with cancer including myelosuppressive therapy;

(e) anaemia associated with rheumatoid arthritis, premature, excessive blood loss, myelofibrosis, sickle cell anaemia, bone marrow transplantation, thermal injury, β -thalassemia, and Acosta's disease; and

(f) diseases in connection with acute or chronic ischemic injury of the myocardium, skeletal muscle cells or renal cells.

56. Use of EPO according to any one of claims 42 to 48 for improving peripheral oxygenation, improving physical performance, facilitating presurgical autologous blood donation, and/or maintaining or increasing hematocrit values in an animal or human body.

57. Use of EPO according to any one of claims 42 to 48 for preventing and treating ischemic acute renal failure, cardiac failure, congestive heart failure, endothelial injury such as inflammation, diseases of the central nervous system, diseases

of the peripheral nervous system, and harmful cell apoptosis or necrosis such as in renal tubular cells myocardial cells, muscle cells, liver cells, bone marrow, and in central nervous tissue such neuronal death in an animal or human body.

- 5 58. Use of EPO according to any one of claims 42 to 48 for inducing, stimulating and/or supporting the formation of new blood vessels, neovascularisation, angiogenesis, vasoproliferative processes, neuroprotection, mitosis, proliferation, cell motility, and wound healing in an animal or human body.
- 10 59. Use of EPO according to any one of claims 42 to 48 as a hormone.
60. Use according to any one of claims 54 to 59, wherein EPO is administered in a dose from 10 IU to 100 000 IU, preferably from 500 IU to 2000 IU.
- 15 61. Use according to any one of claims 54 to 59, wherein EPO is administered in a dose of 0.5 IU to 2000 IU per kg body weight.
- 20 62. Use according to any one of claims 54 to 61 comprising the step of administering EPO in a therapeutically or prophylactically effective dose, in particular in form of a pharmaceutical composition according to any one of claims 42 to 48.
63. Use according to any one of claims 52 to 62, wherein EPO is produced by at least one autologous cell or autologous tissue from an animal or human body, in particular cultured renal

cells, and EPO is administered to the same animal or human body.

64. Use of EPO according to any one of claims 42 to 48 for the preparation of medicament for the treatment or use according
5 to any one of claims 54 to 63.